FISH GENETICS

Robb F. Leary, Ph.D

Division of Biological Sciences
University of Montana
Missoula, Montana 59812

-

U.S. Fish & Wildlife Service
National Conservation Training Center
Course #FIS1102

Table of Contents

Chapter I: Molecular Genetics and Cytogenetics I-1
Introduction I-3
What is DNA? I-3
What is a gene? I-6
What is a chromosome? I-6

Protein Construction I-9
What is RNA? I-9
Genetic code I-14
Summary I-14

Cell Division I-16
DNA replication I-16
Mitosis I-18
Meiosis I-22
Contrast of mitosis and meiosis I-27

Triploids and Gynogenetic Diploids I-28

Problems and Discussion I I-29

Chapter II: Introductory Genetics II-1
Genetic variation II-2
Commonly used terms II-2
Inheritance of single gene traits II-3
Mendelian ratios II-9
Inheritance of multiple gene traits II-11
Heritability II-11

Problems and Discussion II II-16

Chapter III: Introductory Population Genetics and Evolution III-1
Evolution III-2
The nonevolving or equilibrium population III-2
Hardy-Weinberg equilibrium model III-2
Mutation III-7
Natural selection III-8
Migration or gene flow III-11
Genetic drift III-13

Related topics III-16
Founder effect III-16
Bottleneck III-17
Population genetic structure III-17

Problems and Discussion III III-20

Chapter IV: Genetic Variation and Divergence IV-1
Estimating Genetic Variation and Divergence IV-2
Historical views IV-2
Problems IV-2
Solutions IV-2
Protein electrophoresis IV-3
Estimating allele frequencies IV-4
Estimating genetic variation within populations IV-6
Proportion of polymorhic loci IV-6
Average expected heterozygosity IV-6
Average number of alleles per locus IV-7
Estimating genetic divergence among populations IV-8
Similarity or distance estimates IV-8
Principle components\discriminant function IV-8
Partitioning genetic variation IV-10
DNA analysis IV-12
Restriction enzymes IV-12
Polymerase chain reaction (PCR) IV-13
Random amplified polymorphic DNA (RAPD) IV-14
Microsatellites IV-14

Problems and Discussion IV IV-15

Data Interpretation IV-18
Taxonomic questions IV-18
Population genetic structure of a species IV-22
Neutrality IV-24

Importance of Genetic Variation IV-26
Long term IV-26
Short term IV-26

Maintaining Genetic Variation IV-31
Causes of the loss of genetic variation IV-31
Inbreeding IV-31
Directional selection IV-33
Genetic drift IV-33
Establishing a broodstock IV-33
Number of founders IV-33
General IV-33
Considerations from spawning time distributions IV-33
Threatened and endangered species IV-37
Perpetuating a broodstock IV-38
Transferring a broodstock IV-42
Monitoring a broodstock IV-42

Chapter V: Fish Introductions V-1
Genetic Impacts of Fish Introductions V-2
Reasons for introductions V-2
Hybridization/Introgression V-3
Indirect genetic changes due to reduced population size or
changes in selection pressures V-6

Proper Use of Fish Introductions in Population Restoration V-7
Stage I V-7
Stage II V-7
Stage III V-7
Founding new populations of threatened fishes V-7

LITERATURE CITED VI

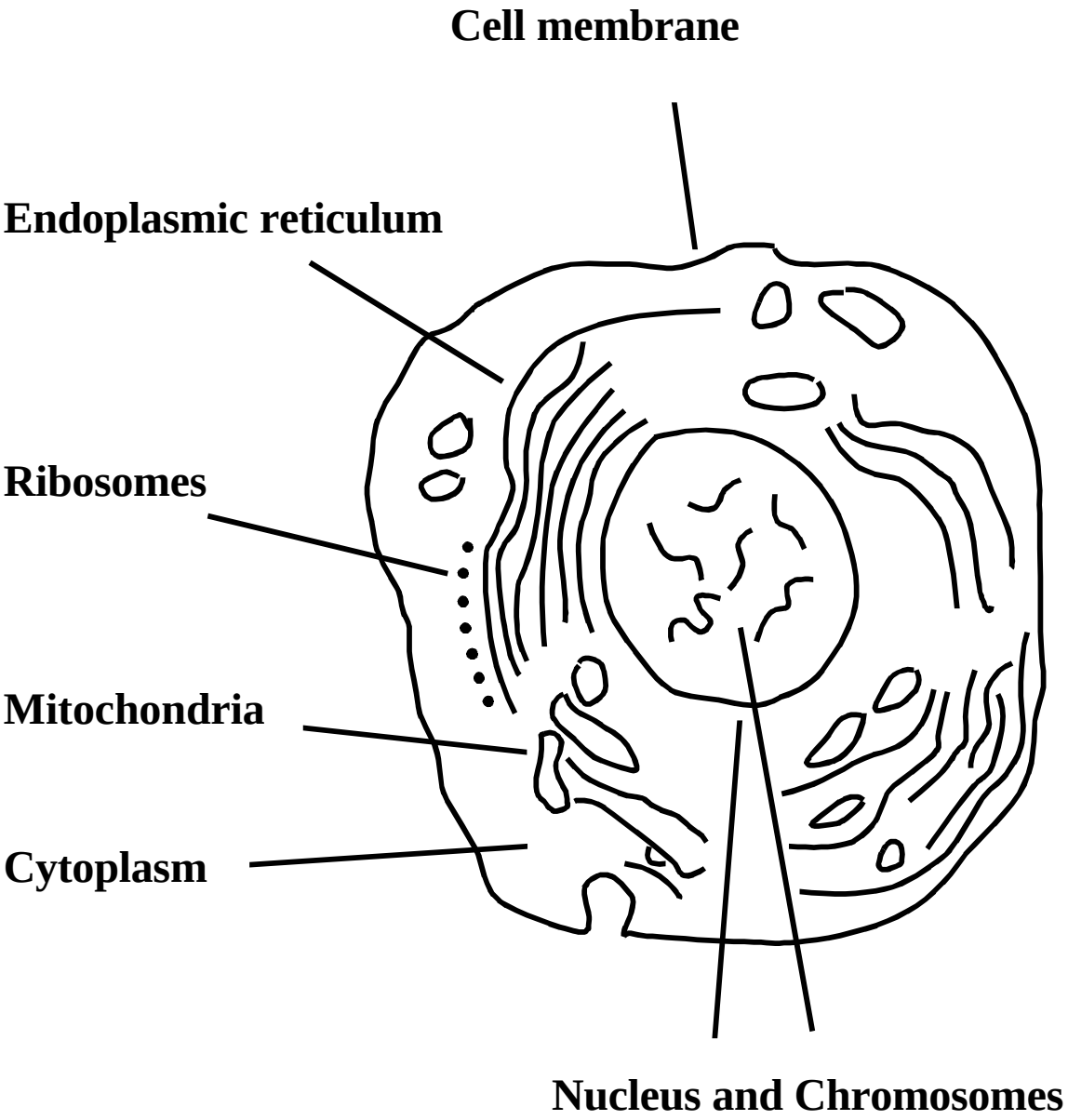
`

GLOSSARY VII

CHAPTER I

MOLECULAR GENETICS AND CYTOGENTICS

Generalized Cell



Introduction

Why be concerned about genetics?

From a genetics perspective organisms can be considered temporary carriers for a set of genes.

Genes not organisms are passed on from generation to generation.

Genetics is the study of how genes are inherited from one generation to the next and how they affect the characteristics of progeny.

What is a **gene**?

A specific segment of a molecule of **deoxyribonucleic acid (DNA)** to which a particular function can be assigned.

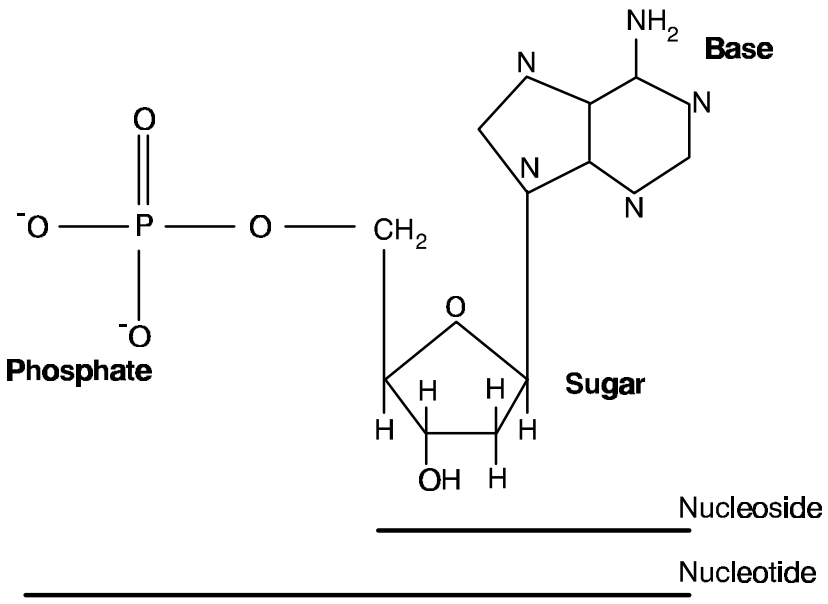
What is **DNA**?

A tightly coiled, helical molecule composed of:

phosphate
sugar: deoxyribose
four different nitrogenous bases
two purines: adenine (A) and guanine (G), 2 rings
two pyrimidines: cytosine (C) and thymine (T), 1 ring
carbon, nitrogen, hydrogen, oxygen

Nucleoside: base plus sugar
Nucleotide: nucleoside plus phosphate (Fig. 1)

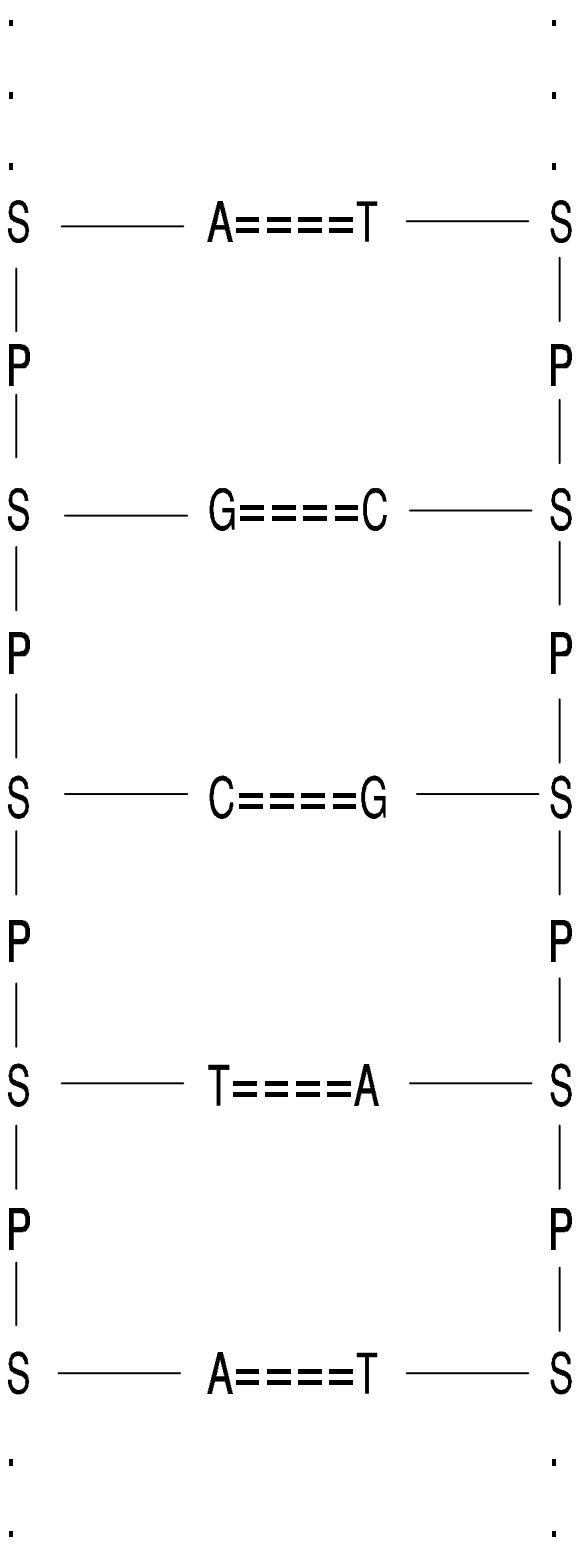
Figure 1



DNA is composed of two strands of **complementary** nucleotides. That is, the sequence of one strand can always be converted to the sequence of the other because A always pairs with T and G with C (Fig.2).

> **Complementarity** is very important. Allows for replication since given one strand you can form the other and allows DNA to act as an information storage and retrieval system.

Figure 2



What is a **gene**?

A specific nucleotide sequence coding for the construction of a protein **(structural gene)** or that regulates the time and place of expression of other genes **(regulatory genes)**.

Genes do not exist as individual units in the cell nucleus, but as a specific part of a chromosome.

What is a **chromosome**?

Single, tightly coiled molecule of DNA in association with protein constituting a linear array of genes.

Occur in pairs in practically all vertebrates.

Members of each pair usually carry the same set of genes because they are derived from a single common ancestor **(homologous)**.

1 **chromosome set**:	**diploid (2N)**
½ **chromosome set**:	**haploid (1N)**
2 **chromosome sets**:	**tetraploid (4N)**
1½ **chromosome sets**:	**triploid (3N)**

Usually divided into two classes:

sex chromosomes: have genes involved in sex determination
autosomes: do not have genes involved in sex determination

Number of chromosomes (2N) is usually highly variable among species but is generally constant from generation to generation within a species (Table 1). 2N can be used for species detection and identification.

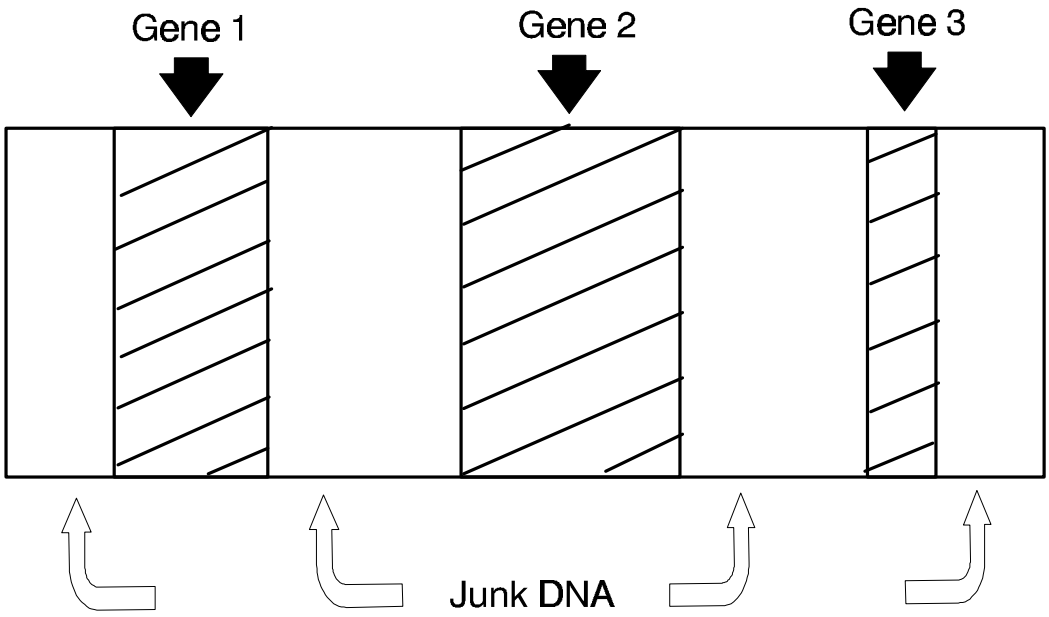
Table 1: Diploid number of chromosomes in madtom catfish (Gold et al. 1980).

Species	2N
Caddo	40
Frecklebelly	42
Tadpole	42
Least	46
Speckled	46
Freckled	48
Brindled	50
Margined	54

The linear array of genes on a chromosome is not continuous.

Adjacent genes are often interrupted by intervening sequences of DNA with no known function ("**Junk DNA**"; Fig. 3).

Figure 3



Genes themselves are often broken into pieces. There are four general parts of a gene (Fig. 4):

upstream flanking region: signals start of a gene

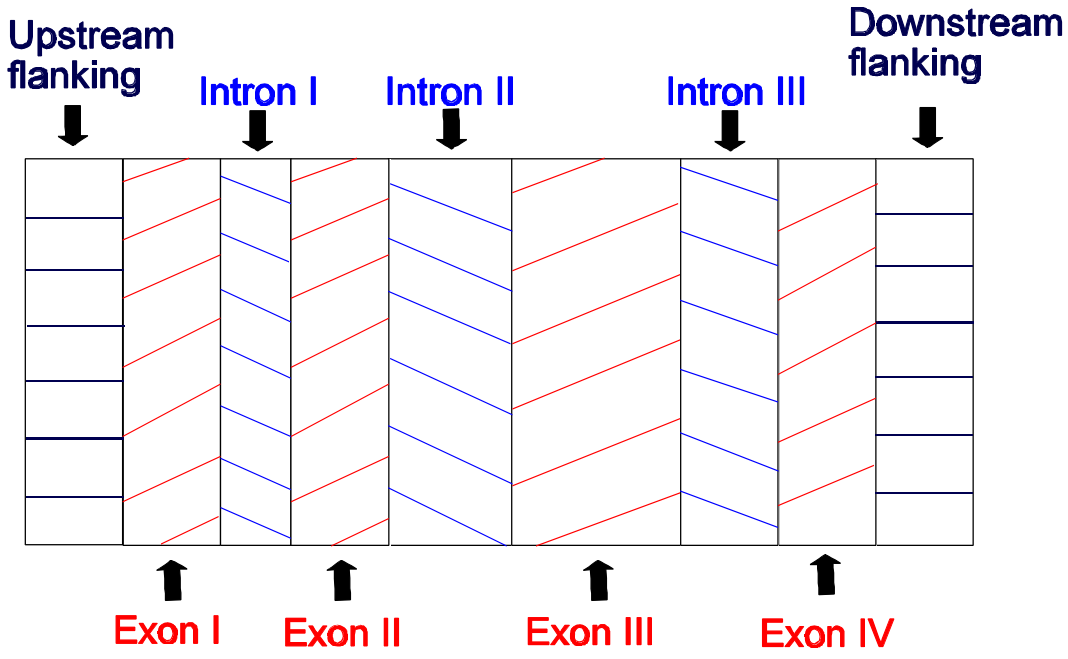
exons: code or perform gene function

introns: intervening sequences between exons

downstream flanking region: signals end of a gene

Introns with no known function are often interspersed among exons, hence genes in pieces.

Figure 4



Protein Construction

Proteins are composed of **amino acids** (Fig. 5). There are many kinds but only 20 are common.

Polypeptide: strand of amino acids formed by bonding of the carboxyl group of one to the amino group of the adjacent amino acid **(peptide bond)** (Fig. 6).

The type and position of each amino acid in a polypeptide represents the **primary structure** of protein.

Nucleotide sequence of a gene determines the primary structure of a protein.

Some polypeptides are proteins but not all proteins are a polypeptide.

Some proteins are composed of two or more polypeptides that may have the same or a different primary structure. Different polypeptides may be coded by the same gene or a different gene; 2 polypeptides from the ∝ and 2 polypeptides from the â hemoglobin genes combine to form hemoglobin.

Quaternary structure: number of polypeptides in a protein.

1 = **monomer**
2 = **dimer**
3 = **trimer**
4 = **tetramer**

How does gene structure determine protein primary structure?

There are three players in the game.

DNA = boss
RNA = workers
protein = product

What is **ribonucleic acid (RNA)**?

A molecule composed of:

phosphate
sugar: ribose
four different nitrogenous bases
two purines: A and G
two pyrimidines: C and Uracil (U)

Different from DNA only because it is single stranded, has a different sugar, and U replaces T.

Figure 5

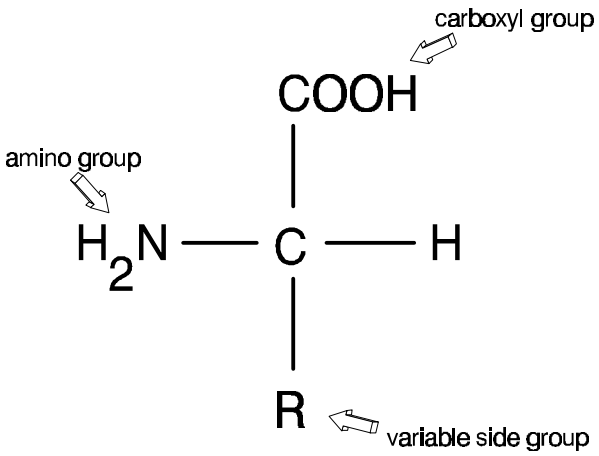
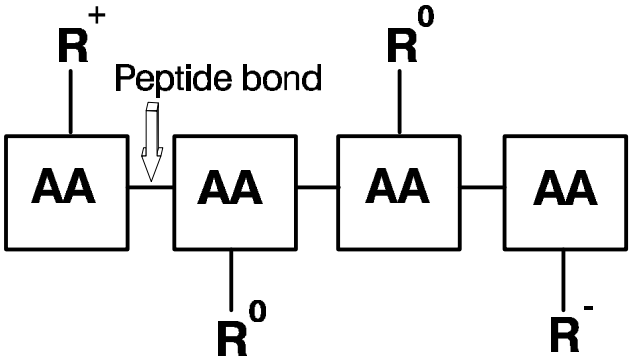


Figure 6



How is RNA made?

It is transcribed directly from a gene.

DNA "unzips" and one strand serves as a RNA template (Fig. 7). Strand transcribed varies from gene to gene but not within a gene.

A strand of RNA complementary to the DNA is made by an enzymatically controlled reaction.

DNA	**RNA**
A	U
G	C
C	G
A	U

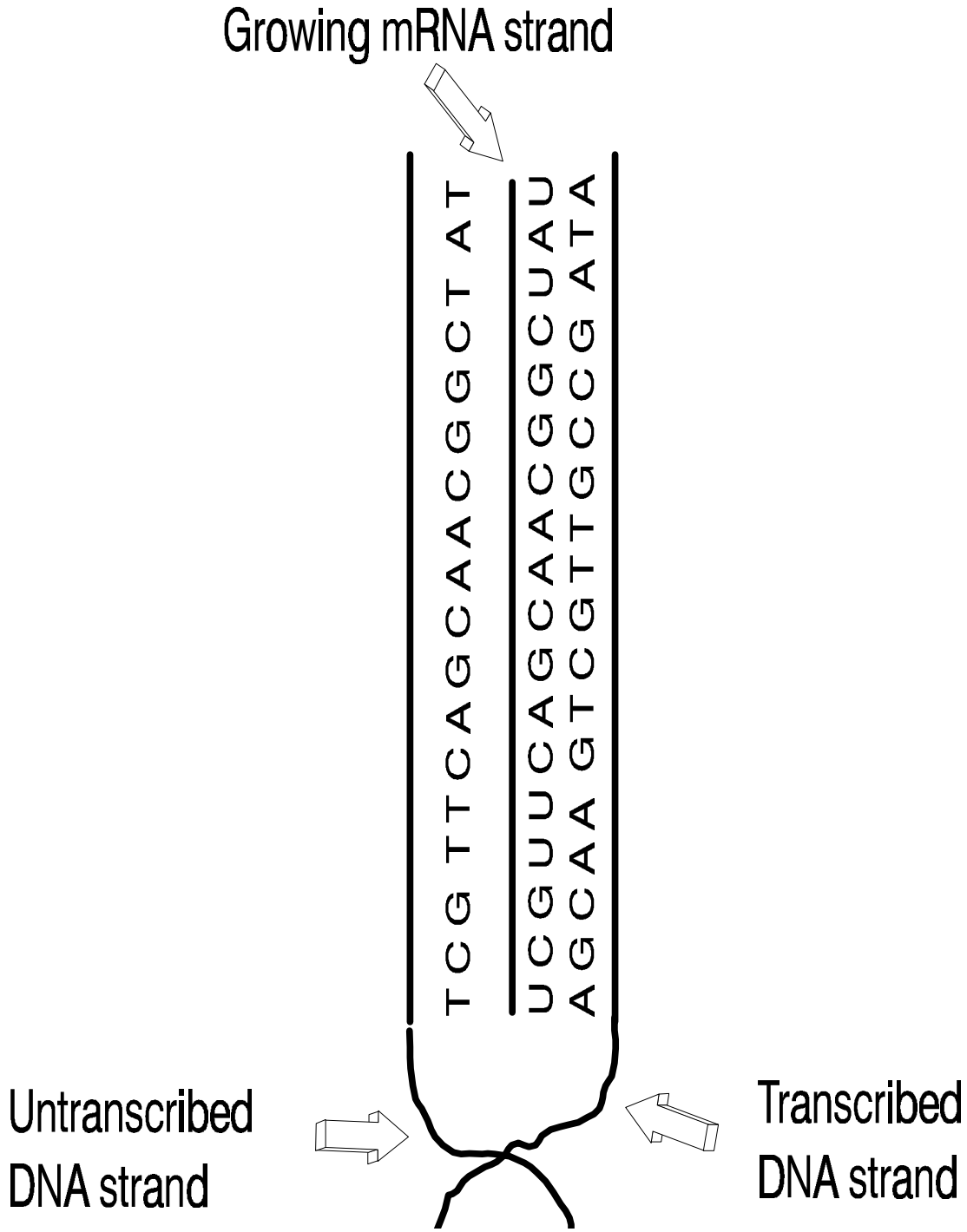
There are three basic kinds of RNA:

Ribosomal (rRNA): are part of the structure of ribosomes, cytoplasmic organelles attached to endoplasmic reticulum that serve as the site of protein synthesis (Cell Diagram, page I-2). Three kinds: 5S, 18S, 28S.

Transfer (tRNA): bond to a specific amino acid and 'carry' to ribosomes for protein construction. Many kinds; at least one for every amino acid.

Messenger (mRNA): brings complement of gene structure from the nucleus to the ribosome where its structure is translated by tRNA to construct a protein. Many, many kinds; at least one for every structural gene.

Figure 7


Growing mRNA strand
T C G T T C A G G C A A C G G G C T A T
U C G U U C A G G C A A C G G G C U A U
A G C A A G T C C G T T G C C C G A T A
Untranscribed
DNA strand
Transcribed
DNA strand

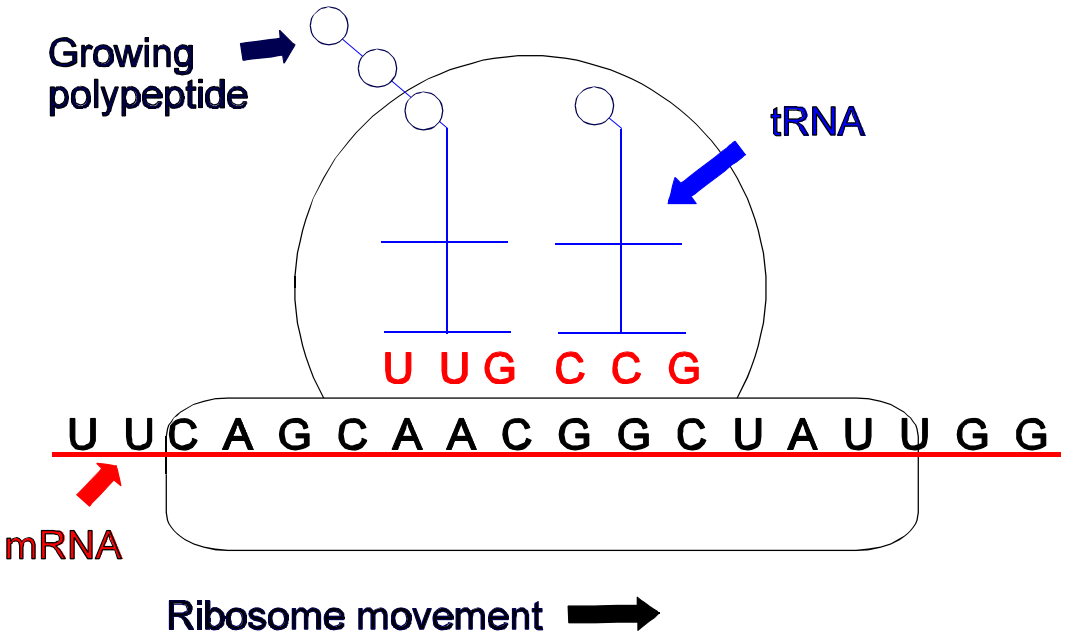
Back to protein construction.

mRNA leaves nucleus and attaches to ribosome in cytoplasm.

tRNA attaches to ribosome and aligns its three nucleotide **anticodon** region with the three nucleotide **codon** region of mRNA.

Amino acids of adjacent tRNA's are joined until entire mRNA message is translated into a polypeptide (Fig. 8).

Figure 8



Genetic code.

Sequence of three mRNA nucleotides (triplet or codon) that code for an amino acid.

Degenerate or redundant because most amino acids are coded by more than one triplet, e.g.,

mRNA codon	tRNA anticodon	amino acid
GUU	CAA	valine
GUA	CAU	valine
GUC	CAG	valine
UUA	AAU	leucine
UUG	AAC	leucine
AUG	UAC	methionine
UGG	ACC	tryptophan

Only methionine and tryptophan have a single codon. Methionine codon initiates polypeptide synthesis.

The code is nonoverlapping.

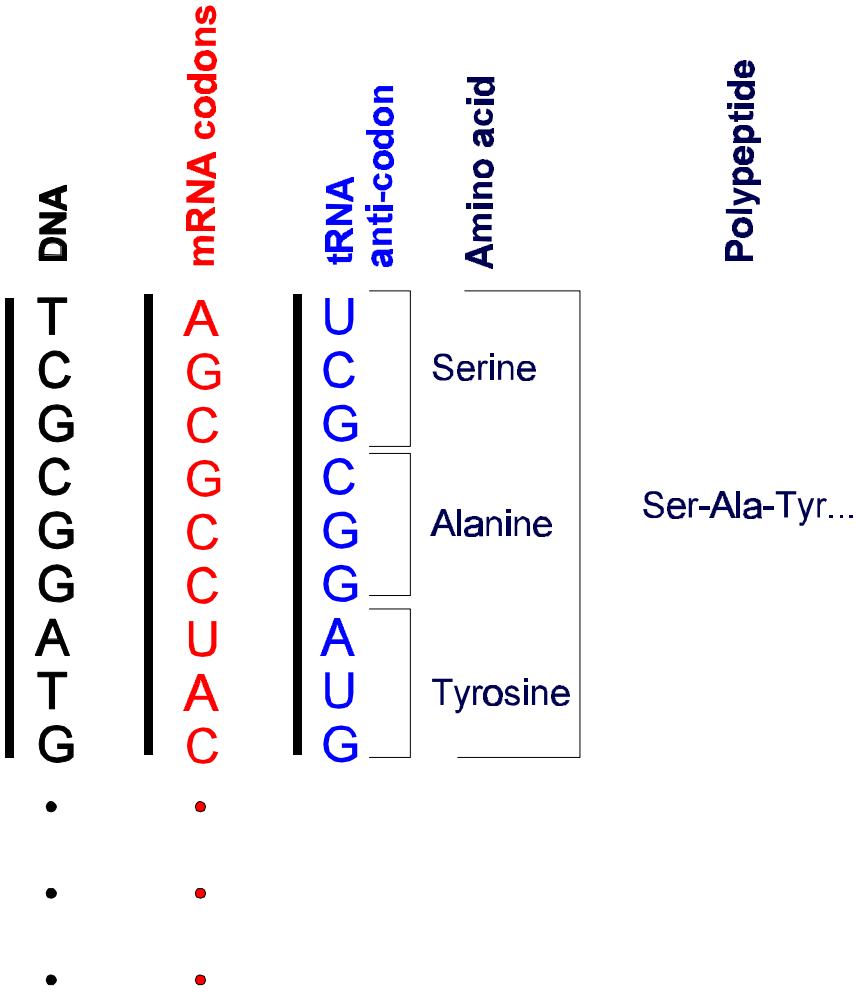
AGCGCCUAG

AGC	GCC	UAG
Codon 1	Codon 2	Codon 3
(serine)	(alanine)	(terminate synthesis)

Summary.

Chromosomes are large molecules mainly composed of a tightly coiled molecule of DNA in association with protein. Genes are a specific segment of DNA of a specific chromosome pair that code for the primary structure of proteins, formation of RNA, or regulate the time and place of expression of other genes. During protein synthesis DNA is transcribed to mRNA and translated to a polypeptide by tRNA on cytoplasmic ribosomes (Fig. 9).

Figure 9

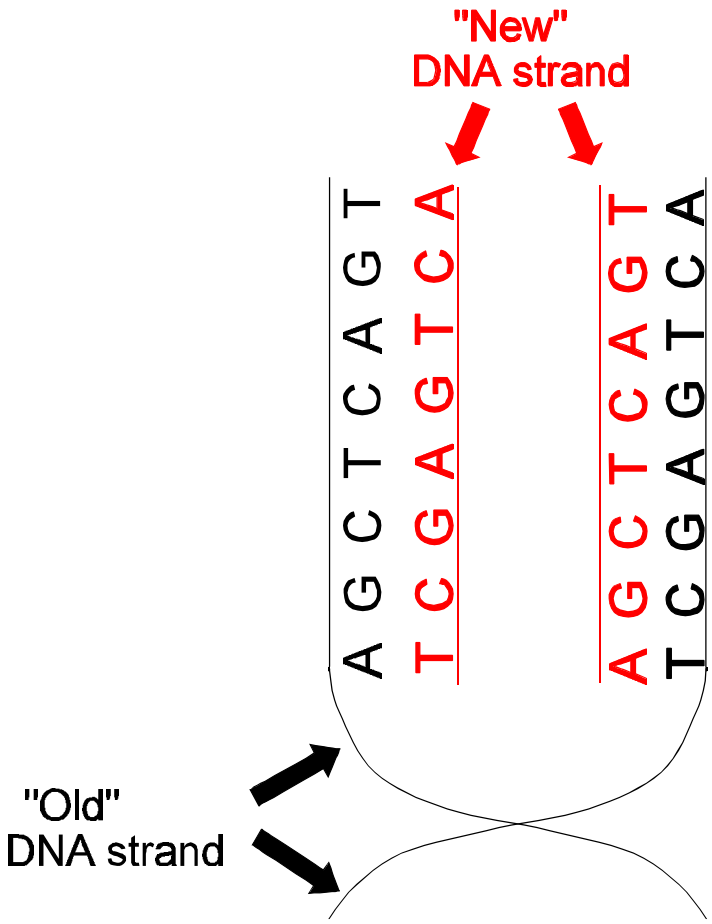

DNA
mRNA codons
tRNA anti-codon
Amino acid
Polypeptide
T C G C G G G A T G
A G C G C C U A C
U C G C G G A U G
Serine
Alanine
Tyrosine
Ser-Ala-Tyr...

DNA replication.

Old strand of DNA serves as a template for construction of a new complementary strand by an enzymatically controlled reaction.

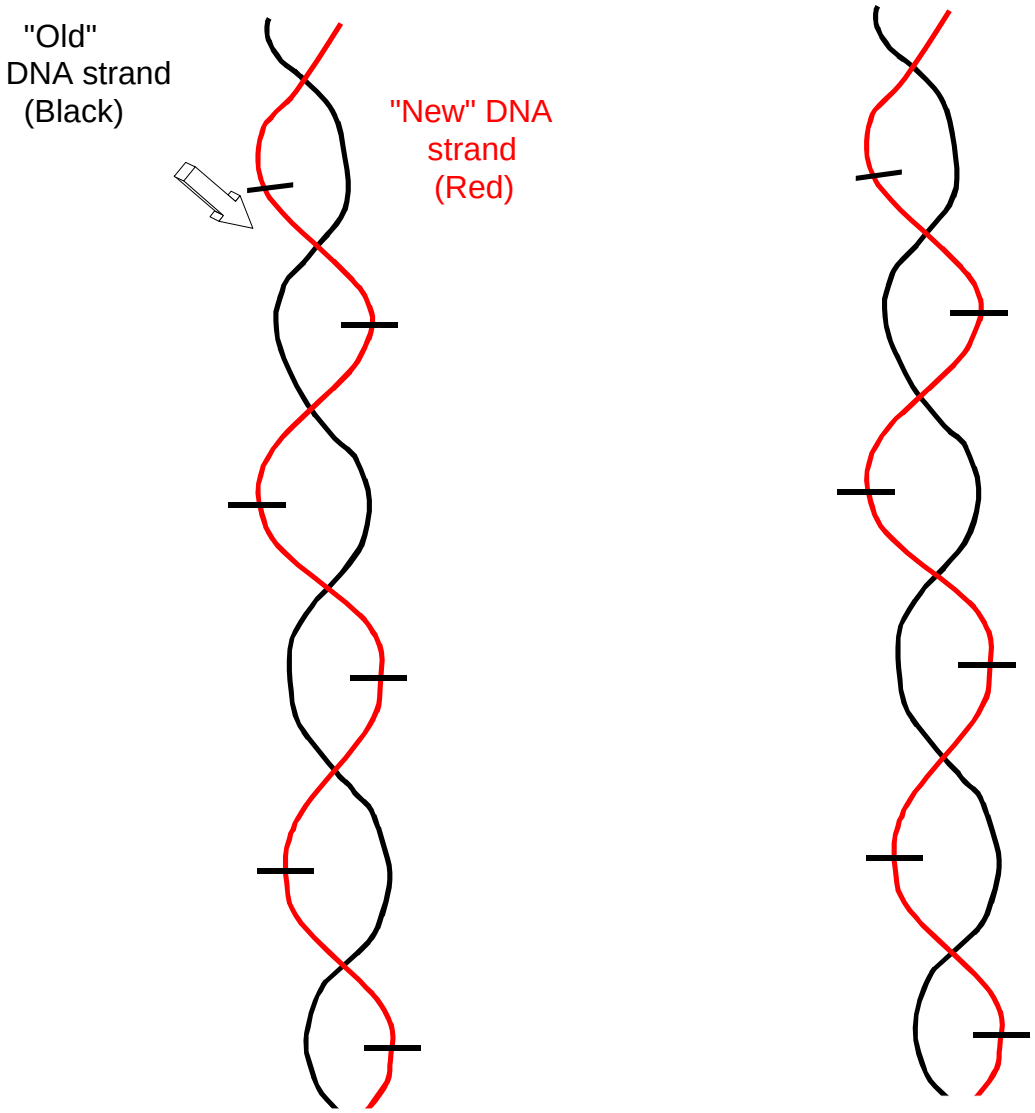
Double helix unzips at multiple sites and replication begins on both exposed strands (Fig. 10).

Figure 10



Semiconservative form of replication because each resulting molecule is half old and half new (Fig. 11).

Figure 11



Two basic types of cell division.

Mitosis: division for body growth, maintenance, and differentiation.

Meiosis: division for the production of **gametes**; eggs and sperm.

Mitosis

Problem: produce one cell from another so they are genetically identical.

Solution: one round of DNA replication followed by one cell division.

Interphase: period of protein synthesis and DNA replication (Fig. 12).

Once fully replicated the replicates or sister strands are physically attached at a region called the **centromere**.

Prophase: chromosomes are fully replicated and begin moving to cell center or equator (Fig. 13).

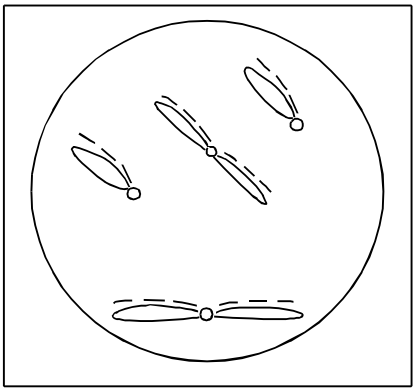
Metaphase: chromosomes align along equator and spindle fibers attach to centromeres (Fig. 14).

Anaphase: sister chromosomes split apart at centromere and are carried to opposite poles by **spindle fibers** (Fig. 15).

Telophase: nuclear membrane reforms and cell divides in two (Fig. 16).

Figure 12

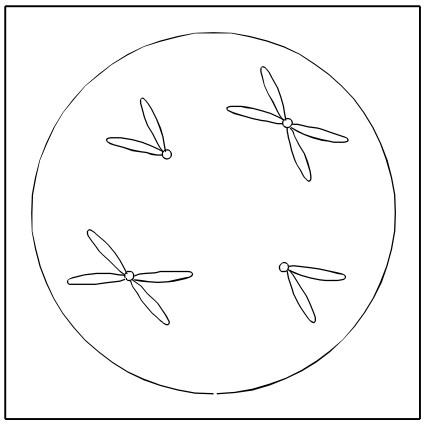
Interphase



Period of protein synthesis and DNA replication

Figure 13

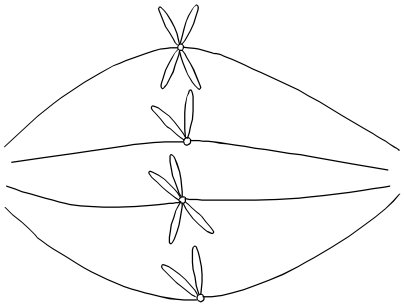
Prophase



Chromosomes are fully replicated and begin to move to cell center or equator

Figure 14

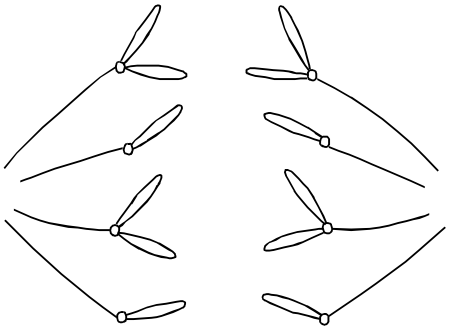
Metaphase



Chromosomes align along equator and spindle fibers attach to centromeres

Figure 15

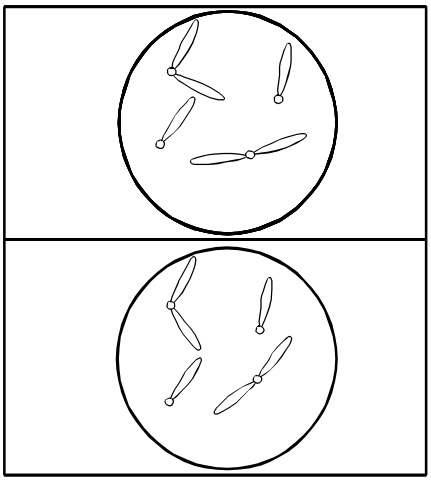
Anaphase



Sisters split at centromere and go to opposite poles

Figure 16

Telophase



Nuclear membrane reforms and
cell divides in two

Meiosis

Problem: keep the amount of DNA constant from generation to generation or to produce a haploid cell. That is, one having only 1N chromosomes.

Solution: one round of DNA replication followed by two cell divisions.

Interphase: protein synthesis and DNA replication.

Reduction Division

Prophase I: homologous pairs of sister chromosomes form and move to equator. Note homologous pairs are not formed in mitosis (Fig. 17).

Metaphase I: homologous pairs align along equator and spindle fibers attach to centromeres (Fig. 18).

Anaphase I: members of each homologous pair move to opposite poles but centromeres do not split (Fig. 19).

Telophase I: nuclear membrane reforms and members of homologous pairs are in different cells but sisters are still attached at the centromere (Fig. 20).

Equational Division

Prophase II: attached sisters in each cell begin to move to equator (Fig. 21).

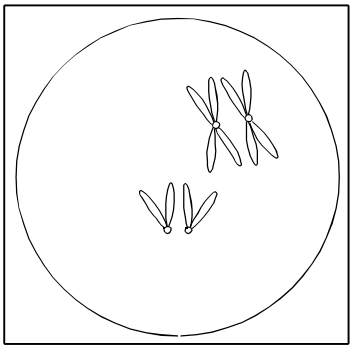
Metaphase II: second alignment at equator and attachment of spindle fibers (Fig. 22).

Anaphase II: centromeres split and sisters move to opposite poles (Fig. 23).

Telophase II: nuclear membrane reforms and each resulting cell is haploid (Fig. 24).

Figure 17

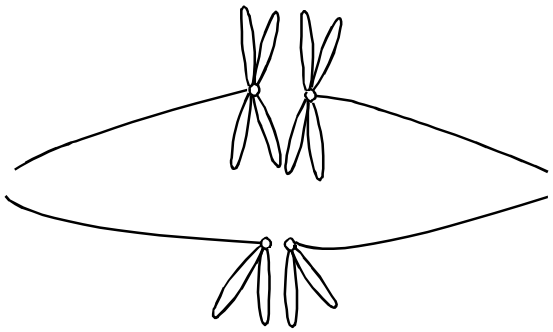
Prophase I



Chromosomes fully replicated, form homologous pairs, and move to the equator

Figure 18

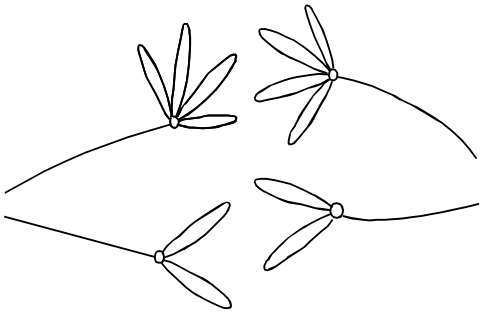
Metaphase I



Homologous pairs align along equator and spindle fibers attach to centromeres

Figure 19

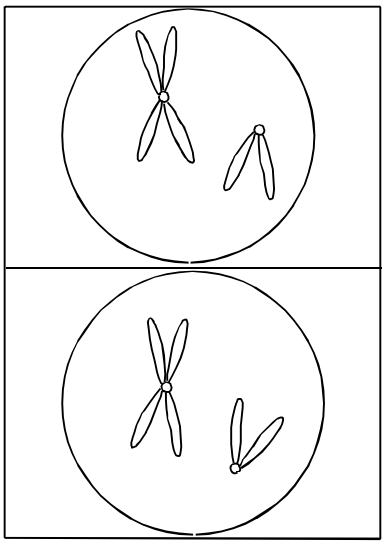
Anaphase I



Members of each homologous pair move to opposite poles: centromeres do not divide

Figure 20

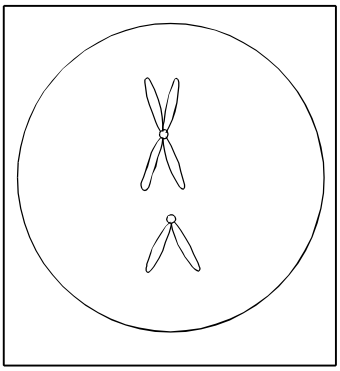
Telophase I



Nuclear membrane reforms but sisters still attached; homologous pairs broken up

Figure 21

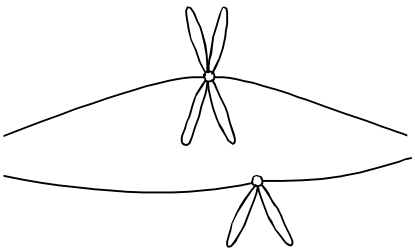
Prophase II



Second movement to equatorial plane

Figure 22

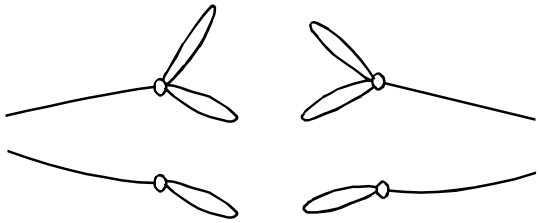
Metaphase II



Second alignment at equator and attachment of spindle fibers

Figure 23

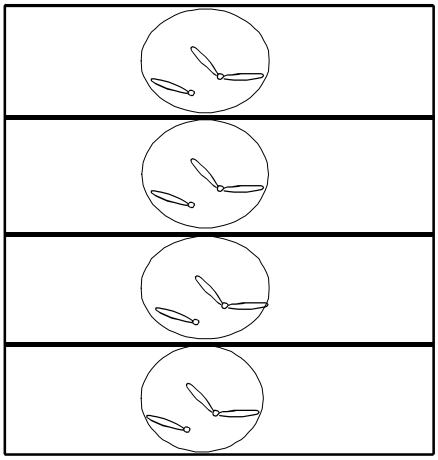
Anaphase II



Centromeres split and sisters move to opposite poles

Figure 24

Telophase II



Nuclear membrane reforms; each cell has only one copy of each pair of chromosomes (haploid)

Sperm Formation: cytoplasm is usually equally divided among daughter cells so each precursor cell yields four sperm cells.

Egg Formation: cytoplasm is usually not equally divided among daughter cells so each precursor yields one egg (almost all the original cytoplasm) and three **polar bodies** (have very little cytoplasm). It is energetically more costly to be female.

Table 2: Contrast of Mitosis and Meiosis

Mitosis	Meiosis
1. Single DNA replication	1. Single DNA replication
2. One cell division	2. Two cell divisions
3. Homologous chromosomes do not pair	3. Homologous chromosomes pair
4. Centromeres divide	4. Centromeres do not divide until second division
5. Chromosome number maintained at 2N	5. Chromosome number halved to 1N
6. Produces two genetically identical cells	6. Produces four genetically different sperm cells or one egg and three polar bodies

Triploids and Gynogenetic Diploids

Green or unfertilized eggs have only completed the first meiotic division; i.e. they are diploid (2N).

Second meiotic division is completed only when egg is fertilized.

Second meiotic division can be prevented using heat shock or pressure.

Triploid (3N) is formed by using heat shock or pressure shortly after fertilization. Result is a diploid egg (2N) fertilized by a haploid sperm (1N). Is not 100% effective, some individuals are diploid. Thus, to release 100% triploids all fish must individually be checked for ploidy.

Gynogenetic diploid is a diploid individual whose chromosomes only came from the maternal parent (mother).

Formed by using sperm subjected to ultraviolet light (UV). UV fragments the DNA so functionally the sperm is 0N.

0N sperm is motile so is used to 'fertilize' eggs and heat shock or pressure shortly after fertilization results in a gynogenetic diploid; 2N egg plus 0N sperm.

Fish are highly inbred so do not constitute a good management tool but are a good research tool.